

SuperSite: dictionary of metabolite and drug binding sites in proteins

Raphael André Bauer, Stefan Günther, Dominic Jansen, Carolin Heeger,
Paul Florian Thaben and Robert Preissner*

Institute of Molecular Biology and Bioinformatics, Structural Bioinformatics Group, Charité- Medical University Berlin, Arnimallee 22, 14195 Berlin, Germany

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ABSTRACT

The increasing structural information about target-bound compounds provide a rich basis to study the binding mechanisms of metabolites and drugs. SuperSite is a database, which combines the structural information with various tools for the analysis of molecular recognition. The main data is made up of 8000 metabolites including 1300 drugs, bound to about 290 000 different receptor binding sites. The analysis tools include features, like the highlighting of evolutionary conserved receptor residues, the marking of putative binding pockets and the superpositioning of different binding sites of the same ligand. User-defined compounds can be edited or uploaded and will be superimposed with the most similar co-crystallized ligand. The user can examine all results online with the molecule viewer Jmol. An implemented search algorithm allows the screening of uploaded proteins, in order to detect potential drug binding sites, which are similar to known binding pockets. The huge data set of target-bound compounds in combination with the provided analysis tools allow to inspect the characteristics of molecular recognition, especially for drug target interactions. SuperSite is publicly available at: <http://bioinformatics.charite.de/supersite>.

INTRODUCTION

The Protein Data Bank (1) contains crystallographic information about proteins, which are co-crystallized with thousands of metabolites or drugs. The data are highly relevant not only for analyzing the recognition of individual compounds (2), but also as a learning set for molecular interaction models (3). In many cases, small compounds bound to macromolecules are medically

active and listed as approved drugs. The consideration of such co-crystallized structures can considerably facilitate the process of drug development (4). Another important aspect of molecular interaction is the specificity of a ligand. Many compounds address several receptor proteins. Comparative analysis of the target proteins can enable to draw conclusions about the molecular recognition between ligands and targets (5). One paradigm, that frequently reoccurs, is the concept of structure activity relationship (SAR)—either meaning, that similar ligands have a similar mode of action (6), or that similar binding sites may share binding partners. This paradigm has implications for finding novel leads, as well as the elucidation of possible side effects (7). SitesBase (8) is an excellent source, which utilizes this similarity concept, using an indexing algorithm, that allows fast comparisons of similar binding sites. This enables the researcher to quickly generate hypotheses about probabilities that a certain binding site will be adopted by a ligand. For further investigations of the interactions between small compounds and macromolecules, a variety of additional sources are available. Concerning experimentally available binding data like K_d , K_i and IC_{50} data, the Binding MOAD (9), PDBbind (10) and the Binding Database (11) are of special interest, since they allow conclusions about the binding affinity of the compounds. Regarding the integration of secondary databases like SCOP (12), CATH (13) and Pfam (14), there is a variety of excellent sources with a strong focus on macromolecules, like PDBsum (15), RCSB PDB (1) and IMB Jena Image Library (16), while PROCOGNATE (17) is especially tailored for elucidating enzymatic activity. However, there is no single resource, which is centered on drug-like compounds, while integrating all available structural information. Therefore, SuperSite was created with three main design goals in mind:

- Rich integration of the PDB, including full-text search, complete 3D information and extraction of ligand–receptor relationships.

*To whom correspondence should be addressed. Tel: +49 30 8445 1649; Fax: +49 30 8445 1551; Email: robert.preissner@charite.de

The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

- Integration of secondary sources, to detect putative binding sites.
- Detection and highlighting of compounds, which are considered to be medically active.

The aim of SuperSite therefore is, to assist the structural biologist with an online tool, which facilitates the inspection of known and putative binding sites, regarding likely binding sites and conservation information. For drug-like compounds, we are additionally providing superimposed binding sites of the same ligand, which allows the detection of structurally conserved residues.

DATABASE AND TOOLS

Primary database

SuperSite's main data source is the PDB, currently containing over 51 000 3D structures and providing well over 290 000 implicit interactions of macromolecules and small compounds. The raw PDB is parsed and translated automatically into a relational database schema that enables SuperSite to further integrate secondary databases for information enrichment (see subsequent subsection). To make the knowledge in the primary database accessible, SuperSite is providing extensible means for querying. The main text query possibilities include the search for PDB-ID, Het-ID, protein, ligand names and synonyms, as well as a full text search, which screens the complete header of all PDB files for a given term. For instance, searching for the term 'insulin' reveals all insulin-related proteins so that they can be used for further investigation. An important subgroup of the proteins in the PDB, are enzymes involved in many catalytic activities. To this end, SuperSite provides an EC tree presentation (18), which makes it possible to browse the PDB via enzyme class/subclass and picking proteins of interest. To investigate the similarity of certain proteins, we have integrated the protein similarity cluster information from the Cd-hit algorithm (18). This information is provided for 95, 90, 70 and 50% similarity, based on the sequence. A specialized search form not only allows the search for similar proteins, but also allows searching for apo-/holo-states. This directly allows to deal with the question, how much the bound form of a protein differs from the unbound form. When it comes to the field of small compounds in the PDB, SuperSite is providing appliances for filtering physio-chemical features like molecular weight, chemical formula or number of atoms. A built-in tool for finding similar small ligands to a given one, is a fingerprint search, based on MyChem fingerprints (<http://mychem.sf.net>). SuperSite also provides Marvin as an online tool (<http://www.chemaxon.com/>), which allows to draw or upload a molecule, and screen it against all ligands contained in the PDB (sdf and mol file formats are supported). User-defined compounds are visualized by a superposition according to the most similar bound ligand.

Secondary data sources

To assist the user in investigating potential binding partners and putative binding pockets, SuperSite integrates

secondary information from related data sources. Analyses of functionally important sites suggest that the degree of conservation within a protein family is a hint for potential binding sites (19). To this end, SuperSite integrates information from HSSP (20), a data source, which contains information about the degree of residue conservation within a family of proteins. As additional source of information, we are providing *de novo* predictions of possible protein binding pockets calculated by LIGSITEcsc (21). This information is precalculated and also stored in the database. HSSP and LIGSITEcsc provide exhaustive information about putative binding sites. Together with the possibility to elucidate related proteins (as described in the subsection above), this provides starting points for the detection of putative binding sites.

Drug site encyclopedia

A subset of all relations, between proteins and small compounds, is the relationship of proteins and drugs. This subset is of high importance, when it comes to a systematic investigation of the desired effects of drugs (on- and off-target effects). An implemented part of SuperSite therefore is the Drug Site Encyclopedia. As the term drug is not self-defining, we compared the World Drug Index (<http://scientific.thomsonreuters.com>), the Comprehensive Medical Chemistry (CMC) Database (<http://www.mdl.com>), the NCI cancer Compounds (<http://dtp.nci.nih.gov>) and SuperDrug (22) with all ligands of the PDB to determine the intersection set using standard fingerprints from OpenBabel (<http://openbabel.org>). The screening was performed via a fingerprint search (<http://mychem.sf.net>). Entities with a Tanimoto coefficient of >0.85 and an equal number of nonhydrogen atoms were considered as drugs (23). This screening yielded more than 1300 medicinal compounds in the PDB. Within the Drug Site Encyclopedia, we are providing extended instruments for exploring the relationship between drug and target. One aspect is the possibility to investigate the superimposed binding sites of the same ligand, showing residues that are conserved in a spatial region, or frequently occur in a region characteristic for drug recognition. Additionally, we are providing a point set match algorithm, which uses known binding sites (patches) of a ligand, to recognize similar patches on the surface of uploaded structures—solved structures or models (algorithm to be published elsewhere). SuperSite is also calculating Lipinski's Rule Of Five (24), reflecting the drug-likeness of uploaded or edited compounds.

Visualization, browsing and availability

SuperSite can be used with a standard web browser with active Java 1.5+. The molecular viewer Jmol (<http://jmol.org>) visualizes proteins, ligands and interactively highlights all integrated data sources like HSSP or LIGSITEcsc. SuperSite also allows browsing between ligand and protein interactions, and vice versa. For instance, it is possible to query the protein 'Insulin', pick out a ligand and jump to the next view providing all co-crystallized proteins. If the ligand is contained in the Drug Site Encyclopedia, it is also possible to investigate the

SuperSite

COMPOUND PYRIDOXAL-5'-PHOSPHATE (HETID: PLP)

Jmol

BINDING SITE AND LIGAND CONFORMATIONS VISUALIZATION

INFO

This window shows all binding sites and all ligand conformation in one coordinate system.

- Explore ligand in SuperSite [show]

SCRIPTING

Enter your jmol script below. The script is also compatible with chime and rasmol scripts. Please use a trailing ";" at the end of your input.
More information at the jmol scripting pages.

OK

BINDING SITE AND LIGAND VISUALIZATION

Note: 1nwy[0] means pdb id 1nwy and binding site / ligand number one (if there are more than one).
In the first column you can select the binding sites / ligands. In the second you can color them.

ID	Binding Site	Ligand
1v2[1]	<input type="checkbox"/> <input checked="" type="checkbox"/>	<input type="checkbox"/> <input checked="" type="checkbox"/>
1c7g[0]	<input type="checkbox"/> <input checked="" type="checkbox"/>	<input type="checkbox"/> <input checked="" type="checkbox"/>
9qpb[0]	<input type="checkbox"/> <input checked="" type="checkbox"/>	<input type="checkbox"/> <input checked="" type="checkbox"/>

Figure 1. Superimposed binding sites of the ligand Vitamin B6 (Het-ID: PLP) from PDB-IDs: 1BJO, 1C7N and 1DJE. Although the proteins show an overall dissimilar structure, residue glycine (red), lysine (blue) and histidine (green) are clustering at specific spatial positions (other atoms of the binding sites depicted in gray).

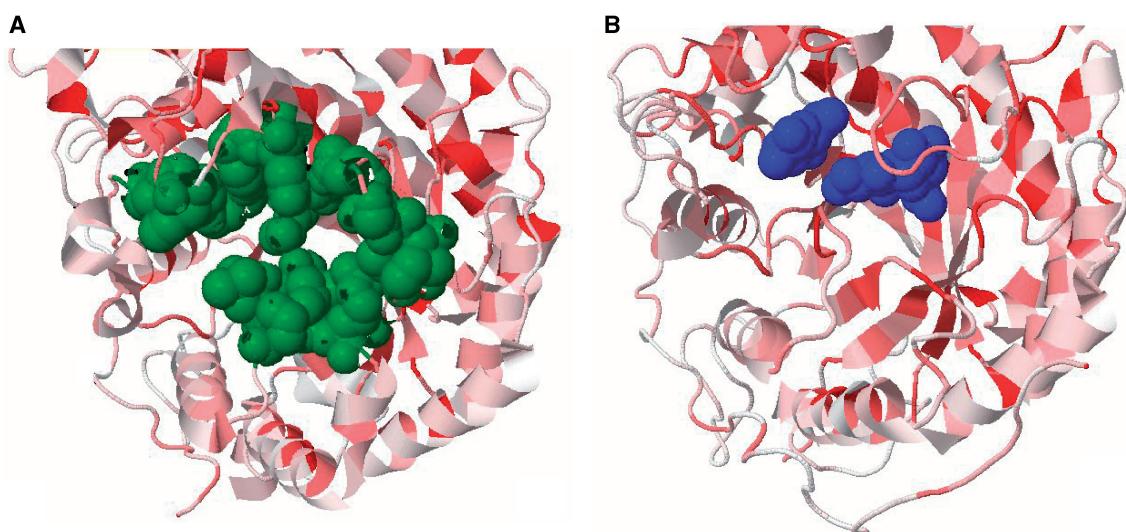


Figure 2. An apo (PDB-ID: 1WDP, **A**) and holo form (PDB-ID: 1B1Y, **B**) of β -amylase. The predictions for the binding site pocket (green) as well as the HSSP conservation (red conserved, white not conserved) support the hypothesis of a binding site at this position. This claim can be proved by the holo form (**B**) with α -D-glucose (blue), bound to the predicted pocket.

superimposed binding sites. Links are provided to numerous relevant sources, containing further specialized data sources [e.g. Proteopedia (25), RCSB, PDBSum]. SuperSite is accessible free of charge for academic institutions. Flat files of the database are available upon request.

CASE STUDIES

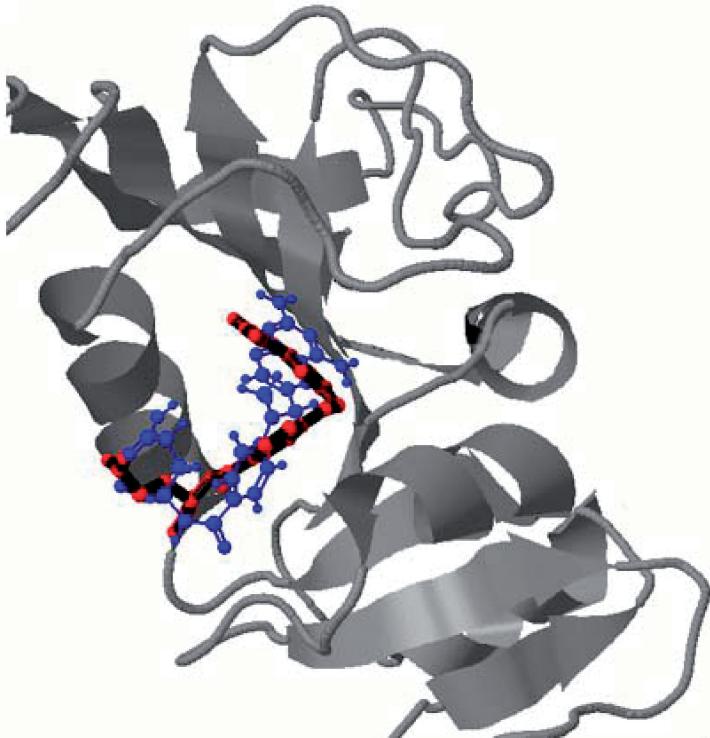
Case study 1: PLP binding partners and spatial mining

Vitamin B6 (Het-ID: PLP) is a co-enzyme, mainly used in the amino acid metabolism and widely present in the human body. Currently, SuperSite contains information



SuperSite

PDB ID: 1RD7



Jmol

LIGANDS IN THIS STRUCTURE

HET_ID	Name/Jmol	Chain	Chain
FOL	FOLIC ACID	A	<input type="button" value="center ligand"/>
FOL	FOLIC ACID	B	<input type="button" value="center ligand"/>
BME	BETA-MERCAPTOETHANOL	B	<input type="button" value="center ligand"/>
BME	BETA-MERCAPTOETHANOL	A	<input type="button" value="center ligand"/>

◀ ▶ ⏪ ⏩ ⏴ ⏵

VISUALIZATION WORKBENCH

VISUALIZATION OPTIONS

- Spacefilling
- Stick_and_Ball
- Cartoon
- Spin on/off
- Show water atoms
- Show ligand atoms

SCRIPTING

Enter your jmol script below. The script is also compatible with chime and rasmol scripts. Please use a trailing ";" at the end of your input.
More information at the [jmol scripting pages](#).



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Figure 3. A dihydrofolate reductase (PDB-ID: 1RA7) with folic acid (HET-ID: FOL, red) bound. One of the highest ranking results from a ligand similarity screening, using compound Methotrexate (Het ID: MTX, blue), suggests a binding at that position.

about 463 structures containing PLP, representing a variety of proteins (e.g. aminotransferases, glycogen phosphorylases). A visualization of all binding sites at once can be achieved, by selecting ‘Drug Encyclopedia’ in the main menu and then entering ‘PLP’ as Het-ID. This view allows inspecting common features, like spatial conservation of specific amino acid types. In the case of PLP, it gets obvious, that, for instance, residue Gly is conserved at a spatial position near the phosphate (Figure 1). This is even the case, when the proteins are structurally dissimilar, a conclusion also discussed in ref. (26).

Case study 2: determination of binding pockets

The elucidation of possible binding pockets and active sites of proteins without co-crystallized compounds is a common task for structural biologists. SuperSite provides two tools for the investigation into this topic: LIGSITEcsc—providing precalculated binding pocket predictions and HSSP—providing information about sequence conservation. For instance, PDB-ID 1WDP refers to the structure of the enzyme β -amylase, solved without substrate. To evaluate if there is a possible binding pocket, the user can consult LIGSITEcsc and HSSP interactively from SuperSite (Figure 2). The HSSP conservation shows a more conserved region around residue glutamine (residue number 186). At the same position, LIGSITEcsc shows a relatively large predicted binding pocket. There is another β -amylase (PDB-ID: 1B1Y) similar in overall structure to the apo form containing a ligand at the position proposed by LIGSITEcsc and HSSP what shows the applicability of this method.

Case study 3: detection of binding partners via similarity screening

SuperSite also offers a facility for the fast similarity screening of a compound, against all ligands co-crystallized in the PDB. This enables to hypothesize about possible binding partners for similar compounds. Methotrexate (Het-ID: MTX) is a drug, which is used as anti-inflammatory agent/immunosuppressant and in high concentrations used as chemotherapeutical agent (27). Methotrexate inhibits the folic acid biosynthesis and therefore slows the proliferation of cells. SuperSite enables the user to find similar compounds in the PDB, by simply drawing, or by uploading a mol or sdf file. After issuing the similarity search for Methotrexate, one of the best hits not identical to Methotrexate, is folic acid (HET-ID: FOL) bound to a dihydrofolate reductase (Figure 3). The query compound Methotrexate is superimposed with folic acid, which is the known mode of action.

CONCLUSIONS

We presented a novel database, SuperSite that offers 3D information about proteins and about their bound compounds (ligands). SuperSite enables the user to investigate into the relationship of ligand and receptor in atomic detail, integrating information sources about putative binding sites and conservation on residue level. SuperSite is made with an emphasis on ligands, that are drug-like and

therefore of special interest for medical research. To this end, SuperSite provides 3D superpositions of all binding sites of a certain ligand, which enable the user to investigate into the spatial arrangement and properties of the binding site. For further investigations, SuperSite allows to issue a similarity screening against ligands bound in macromolecules as well as a screening of proteins against known binding sites. SuperSite is publicly available at: <http://bioinformatics.charite.de/supersite>.

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